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In Silico Analysis of *CatSper* Family Genes and APOB Gene Regulation in Male Infertility

Sujata Maurya, Nihar Ranjan Bhoi, Kavindra Kumar Kesari, Shubhadeep Roychoudhury, and Dhruv Kumar

Abstract

Sperm concentration and sperm motility are the two major causes of male infertility. Having spermatozoa in semen without motility or flagellum tail defect is a major concern needed to be investigated. The *CatSper* genes are the novel family of four sperm-specific Ca^{2+}-permeable channels which plays an important role in sperm motility, acrosome reaction, sperm, and oocyte fusion. *CatSper1*, *CatSper2*, and *CatSper3* are very well-studied genes for their role in asthenozoospermia, but the association of these genes with metabolic genes is still unstudied. Another unrevealed aspect is how ROS alter the function of *CatSper* genes. Among the *Catsper* family genes, the role of *CatSper4 gene* must be explored more. In this study, we have used the in silico approach to find the connection between the *CatSper* family gene with glycolytic genes and also the involvement of CATSPER4 protein in sperm flagellum using the STRING database. Connection of CATSPER1 protein with lipid metabolic gene is also found in Reactome database, and after that gene ontology of these genes was done by using DAVID and Enrichr databases. This analysis showed a strong interaction between CATSPER1, CATSPER2, and CATSPER3 protein with glycolytic protein (i.e., GAPDHS and PGK2), and CATSPER4 protein shows strong relation in the function of sperm flagellum. We also found a novel gene, i.e., APOB contributing to sperm motility. Gene ontology showed the role of APOB and glycolytic proteins in sperm motility. Enrichr analysis showed the association of APOB and glycolytic proteins in asthenozoospermia and CATSPER4 protein with sperm flagellum. Elsevier Pathway Collection also showed proteins involved in male infertility (i.e., GAPDHS). Therefore, we report the role of the *CatSper4* gene in sperm tail function and the APOB novel gene involved in sperm motility. Understanding the molecular mechanism(s) of regulations of the *CatSper* family gene will

S. Maurya · D. Kumar (✉)
School of Health Sciences & Technology, UPES University, Dehradun, Dehradun, Uttarakhand, India

N. R. Bhoi
Indira IVF Hospital, Indira Fertility Academy, Udaipur, Rajasthan, India

K. K. Kesari
Department of Applied Physics, School of Science, Aalto University, Espoo, Finland

S. Roychoudhury
Department of Life Science and Bioinformatics, Assam University, Silchar, Assam, India

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help us to develop new therapeutic approaches in infertile males.

Keywords

Sperm motility · Sperm flagellum · *CatSper* genes · Glycolytic genes · Lipid metabolic genes · Asthenozoospermia · Male infertility

18.1 Introduction

In the past few years, male infertility has increased rapidly by each year because of advanced age marriages, bad lifestyle, fast food consumption, smoking, drinking alcohol, environmental pollutions, psychological hazards, and many more factors (Kumar and Singh 2015). According to the World Health Organization (WHO), 15% of the couples of childbearing age are suffering from infertility worldwide, in which the male partner contributes 50% of overall cases. Infertility stands third most difficult disease globally after cardiovascular disease and cancer (https://www.who.int/news-room/fact-sheets/detail/infertility). Reduced sperm count and low sperm motility are the two most common causes of male infertility. According to WHO manual sixth edition, sperm motility is divided into four-category classification, i.e., rapidly progressive, ≥25 μm/s or at least half tail length per second (normal sperm); slowly progressive, 5 to <25 μm/s or at least one head length to less than half tail length/sec, and nonprogressive, <5 μm/s, or less than one head length (asthenozoospermic sperm). Immotile: no tail movement and also called sever asthenozoospermia.

Calcium ion plays an important role in sperm motility, capacitation, and acrosomal reaction. The influx of calcium ion is regulated by *CatSper* channels which is situated in plasma membrane of sperm mid-piece (Lishko et al. 2012). Ca^{2+} further helps in generation of cAMP which leads to activation of tyrosine phosphorylation which causes hyperactivated progressive motile sperm (Jin and Yang 2017). But due to aging, reactive oxygen species (ROS) start generating in spermatozoa which not only oxidize cholesterol but also inhibit the activity of tyrosine phosphorylation which lead to immotility or less motility in sperm flagellum (Fig. 18.1) (Pereira et al. 2017). Defects in the mammalian flagellum (sperm tail)

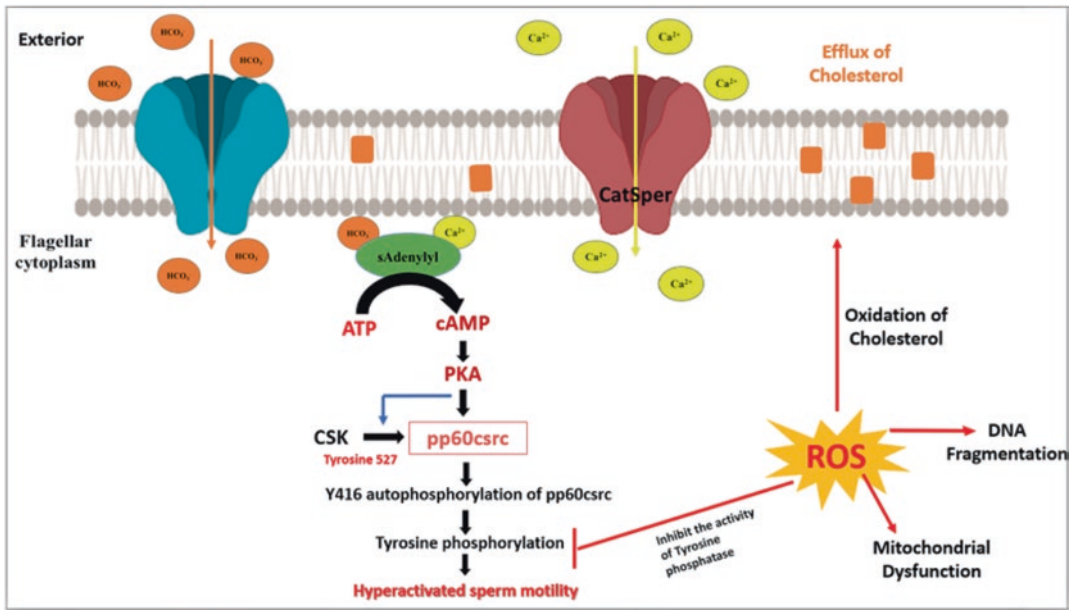


Fig. 18.1 Signaling pathway shows ROS to induce tyrosine phosphorylation in the fibrous sheath of the sperm tail that reduce sperm motility or cause immotility of sperm

which is a specific type of motile cilium result in reduced sperm motility or immotility. Therefore, understanding the mechanism involved in the formation of the sperm tail and knowing its function are needed for solving the issues regarding male infertility (Aprea et al. 2021). At the last phase of spermatogenesis, haploid round spermatids differentiate during spermiogenesis. Spermiogenesis is a process where the nucleus is condensed, the acrosome and sperm tail are formed, and excess cytoplasm is discarded. It involves 16 steps in which the first 8 (1–8) stages include the appearance of a round nucleus, flattening of acrosome, and beginning of elongation of the axoneme from the distal centriole (O'Donnell 2015).

As flagella contain a 9 + 2 microtubule structure similar to motile cilia, therefore, they require the same molecular mechanisms for their formation. At the later (9–14) stages, a transient microtubular platform, the manchette, surrounds the distal part of the sperm head, participating in shaping the head and delivering the proteins to the developing tail (Lehti and Sironen 2016). During the last steps of spermiogenesis, mitochondria are assembled helically around the outer dense fibers (ODFs) in the mid-piece of the sperm tail. While dynein arms in the axoneme provide the motor force for sperm tail motility, all accessory structures are required for the efficient fertilization capacity of sperm. They stabilize the long axoneme and provide support for the sperm tail movement and metabolic pathways for energy production (Linck et al. 2016).

Recent studies on human spermatozoa revealed the association of more than 1000 proteins with the sperm tail structure. This kind of study tells us about the complexity of flagellum and about the genes that cause asthenozoospermia. 26% of identified proteins are found to be associated with lipid metabolism and energy production (Amaral et al. 2014). Therefore, in this study, public database resources and computational tools were used to investigate the genes involved in the energy-driven pathway and lipid metabolism, which led to sperm tail defect in males. This research also aims to elucidate the molecular mechanism(s) of these genes to develop therapeutic approaches in infertile males.

18.2 Materials and Methods

18.2.1 Identification of Asthenozoospermia Gene Targets

Genes associated with "asthenozoospermia," "low sperm motility," "no sperm motility," and "dysregulated sperm motility" were collected from existing databases and literature. Databases like GeneCards (https://www.genecards.org) and DisGeNET (http://www.disgenet.org) were used. The GeneCards database provides comprehensive, user-friendly information on all annotated and predicted human genes (Safran et al. 2010). The DisGeNET database is a discovery platform containing one of the largest publicly available collections of genes and variants associated with human diseases (Piñero et al. 2020).

18.2.2 Protein-Protein Interaction (PPI) Network Analysis

The common genes that were collected from GeneCards and DisGeNET were put into the STRING database (https://string-db.org/,version 11.0) for PPI network analysis. The interacting proteins with a confidence score of $\geq$0.900 were chosen for PPI network visualization construction (Szklarczyk et al. 2019). The proteins that show positive correlation with glycolysis gene, sperm flagellum, and spermatogenesis were chosen for pathway analysis and gene ontology.

18.2.3 Pathway Analysis and Gene Ontology (GO)

Reactome Pathway database (https://reactome.org) was used for understanding the metabolic pathways in which selected genes are involved (Haw et al. 2011). For gene ontology Enrichr tool

(https://maayanlab.cloud/Enrichr/) and DAVID bioinformatic database (https://david.ncifcrf.gov/tools.jsp) were used to get the information on the functions of genes and to understand the biological meaning behind the gene set (Kuleshov et al. 2016; Dennis Jr et al. 2003; Huang et al. 2007). According to a high count and $P < 0.05$, the GO terms and pathways were selected that validate the association of chosen genes with male infertility.

18.3 Results

Total nine genes CatSper1, CatSper2, CatSper3, CatSper4, GAPDHS, PGK2, LDHC, PGM4, and PGM2 were selected from literature review and databases that show its association with asthenozoospermia or with severe asthenozoospermia.

18.3.1 PPI Network Analysis

The selected nine potential targets were input into the STRING database to construct a PPI network. In the network, the nodes and edges represent proteins and protein-protein associations, respectively. In this CATSPER1, CATSPER2, and CATSPER3 show their interaction with glycolytic proteins (i.e., GAPDHS and PGK2), and CATSPER4 protein shows its involvement in sperm flagellum (Fig. 18.2).

18.3.2 Pathway Analysis

In Reactome database CATSPER1, CATSPER2, and CATSPER3 protein were selected to elucidate their role in metabolic pathway. It was found that CATSPER1 protein was involved in three metabolic pathways (Fig. 18.3), and further analysis also showed the interaction of APOA2 gene involved in lipid metabolic pathway with CATSPER1 protein with score 0.781 (Fig. 18.4).

18.3.3 PPI Network Analysis of Selected Genes with APOA2 Genes Involved in Lipid Metabolism

After finding the interaction of CATSPER1 protein with lipid metabolic gene, again PPI analysis was done, and it was found that APOB (gene involved in lipid metabolism) shows its association with flagellated sperm motility and spermatogenesis in human (Fig. 18.5).

18.3.4 Gene Ontology

To validate the findings from PPI network and pathway analysis that gene ontology was done, Enrichr and DAVID databases showed the association of APOB with asthenozoospermia (Fig. 18.6) and sperm motility, respectively (Fig. 18.7), and in Enrichr tool it was also found that GAPDHS (glycolytic protein) is involved in male infertility with *p*-value 3.502e-^{8} (Fig. 18.8) and CATSPER4 protein is found in sperm flagellum with *p*-value 0.0030 (Fig. 18.9).

18.4 Discussion

Functional spermatozoa are important in male fertility and are produced by going through complex processes like meiosis, mitosis, spermatogenesis, and spermiogenesis (Lehti and Sironen 2017). They are highly specialized cells with distinguishing functional region; therefore the expression of key genes is required to regulate the completion of spermatogenesis successfully (O'Donnell et al. 2017; Cheng and Mruk 2012). We hypothesized that alternative expressions of the selected genes *CatSper1*, *CatSper2*, *CatSper3*, *CatSper4*, GAPDHS, PGK2, LDHC, PGM4, PGM2, APOA2, and APOB would contribute to dysregulated sperm motility and defective sperm tail. This study performed using bioinformatics data mining

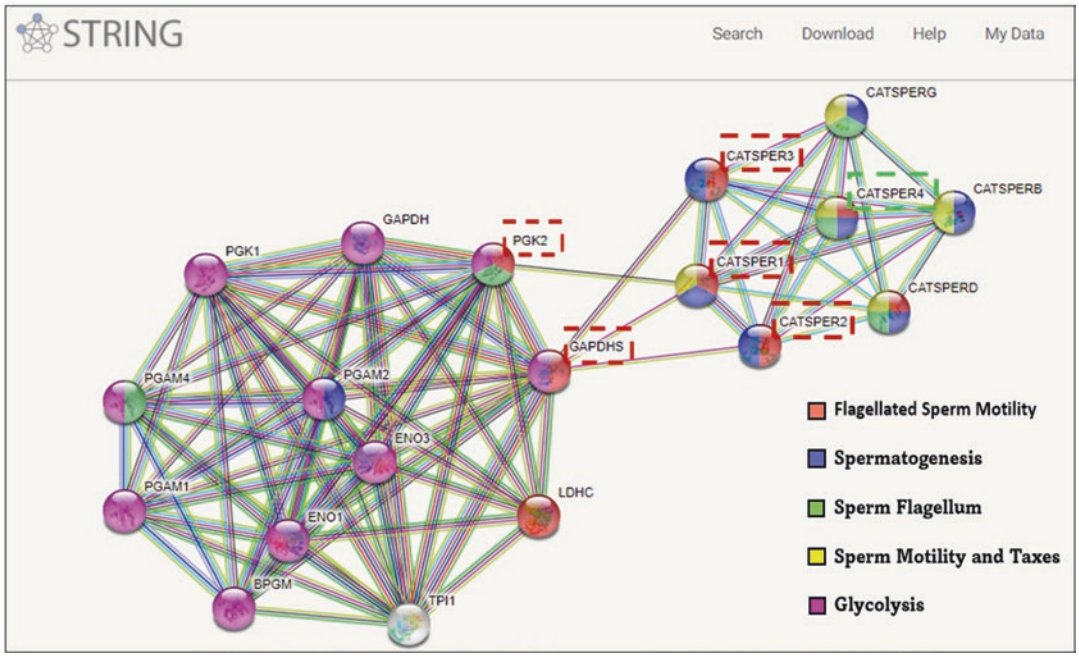


Fig. 18.2 PPI network analysis of nine selected genes, in which red color indicates flagellated sperm motility, the blue color indicates spermatogenesis, green color indicates sperm flagellum, yellow color indicates sperm motility and taxes, and pink color indicates glycolysis

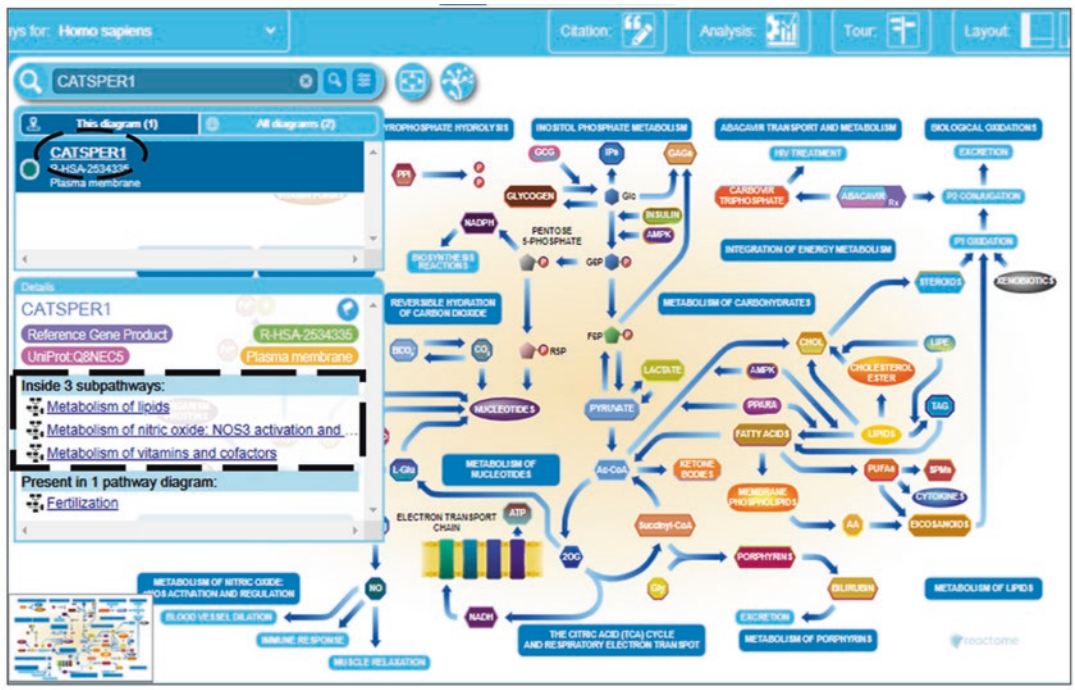


Fig. 18.3 Expression of CatSper1 gene in three different metabolic pathways. (1) metabolism of lipids; (2) metabolism of nitric oxide: NOS3 activation; (3) metabolism of vitamin and cofactors

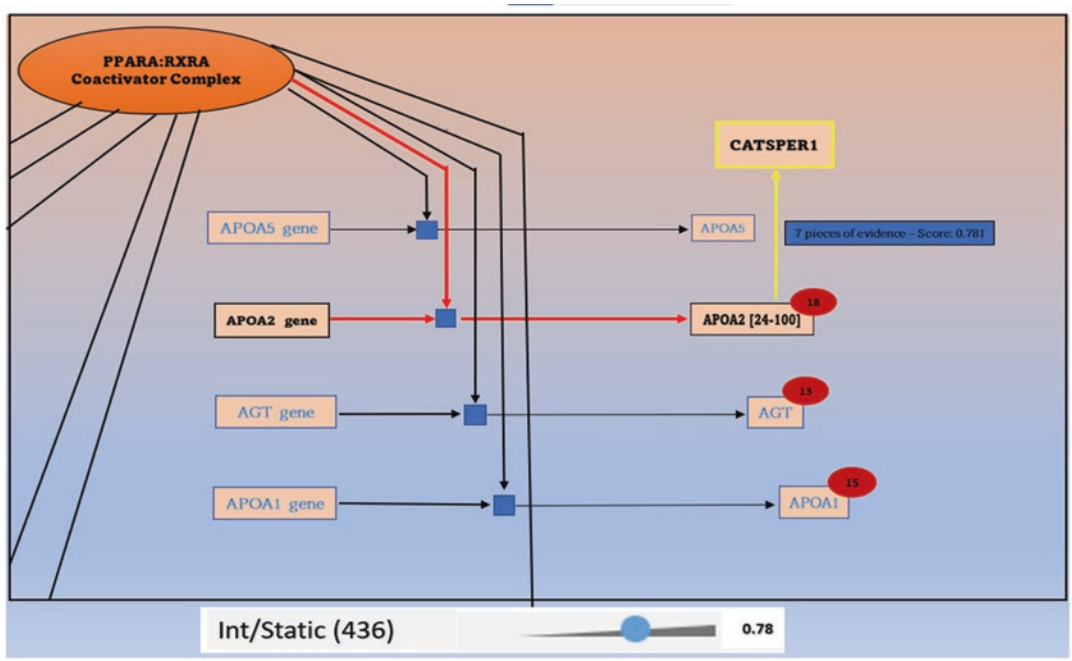


Fig. 18.4 Gene involved in lipid metabolism (APOA2) showed its interaction with CatSper1 gene with an interacting score 0.78

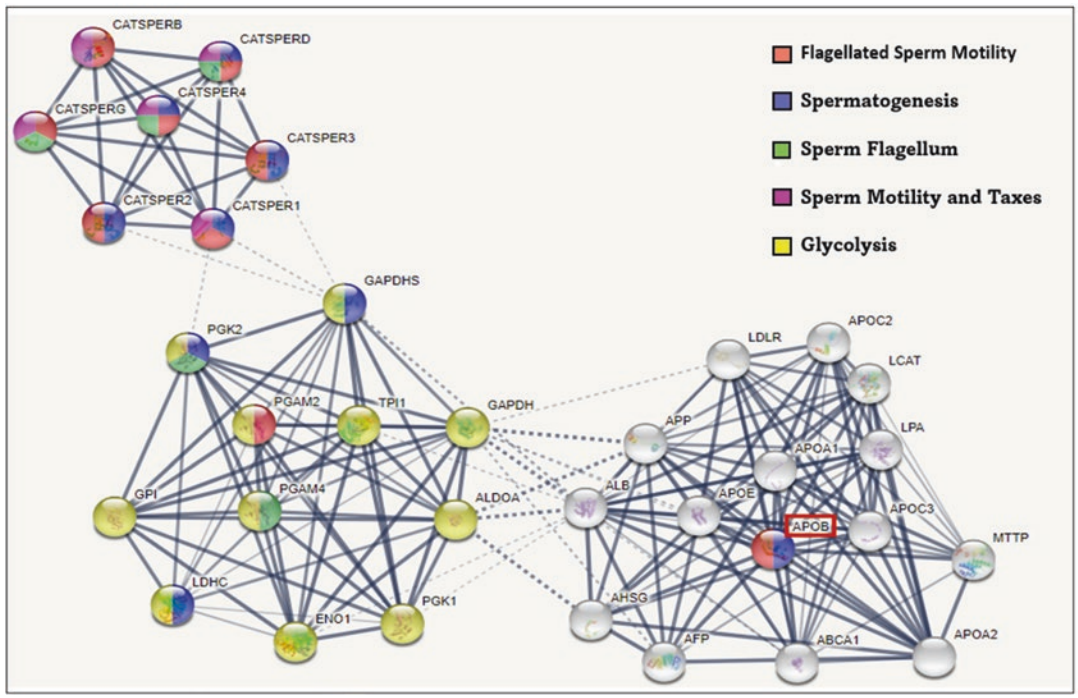


Fig. 18.5 PPI analysis that shows the association of APOB in sperm motility and spermatogenesis, in which red color indicates flagellated sperm motility, the blue color indicates spermatogenesis, green color indicates sperm flagellum, yellow color indicates glycolysis, and pink color indicates sperm motility and taxes

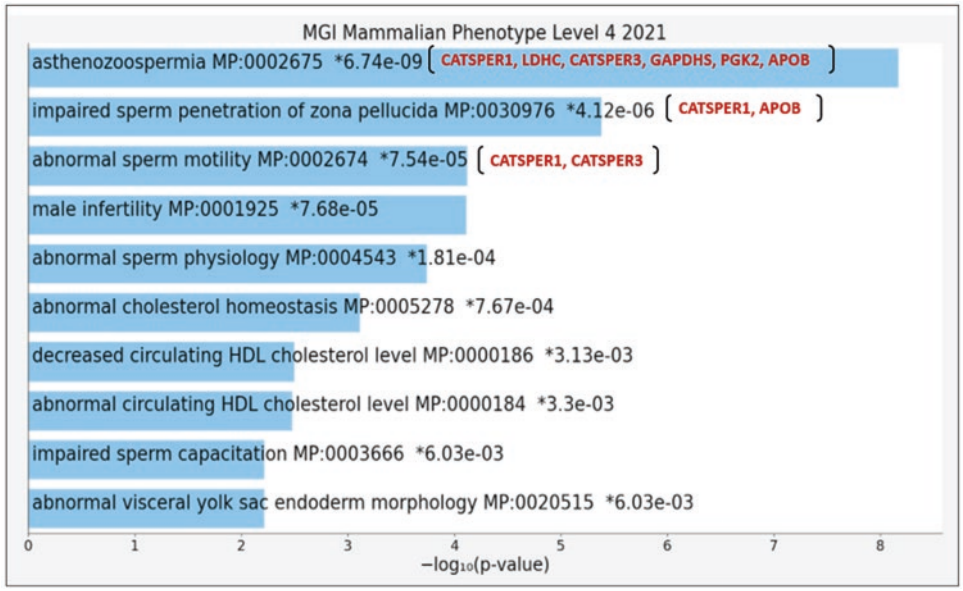


Fig. 18.6 Enrichr result showing the involvement of glycolytic genes and APOB in asthenozoospermia with *p*-value 6.74e-^{09}

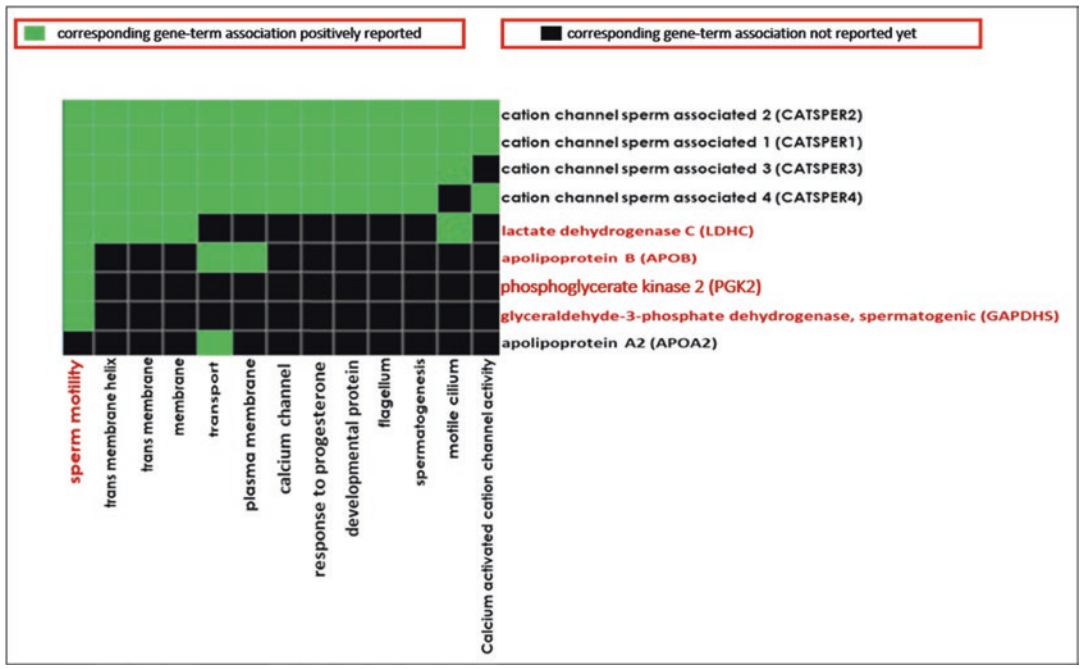
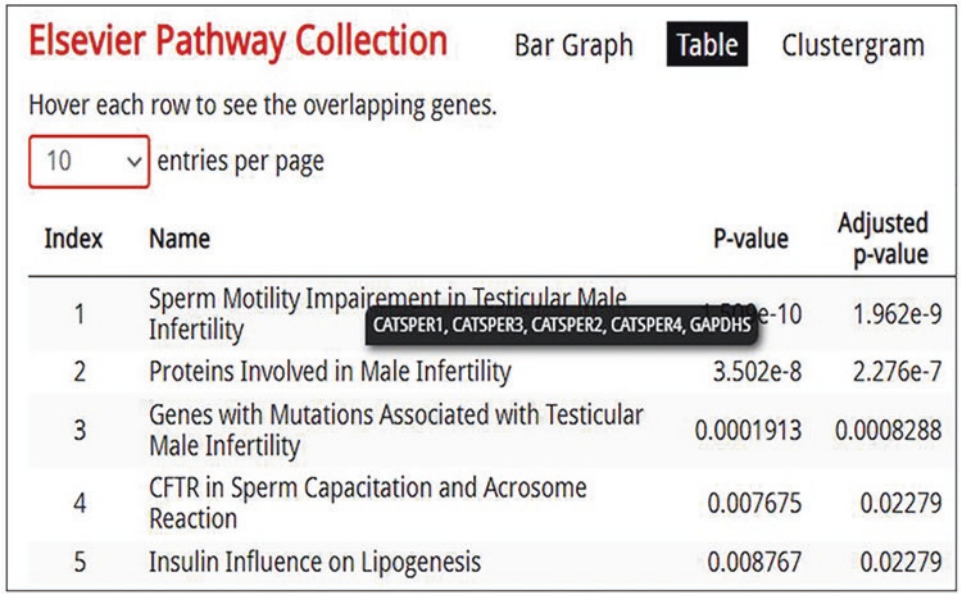


Fig. 18.7 Result of functional clustering from DAVID database showing involvement of glycolytic genes and APOB in sperm motility

of genes associated with asthenozoospermia and male infertility in humans provided a novel insight into the understanding of sperm motility. In the present study, we performed a systematic bioinformatics analysis by collecting genes associated with asthenozoospermia. Total nine genes were



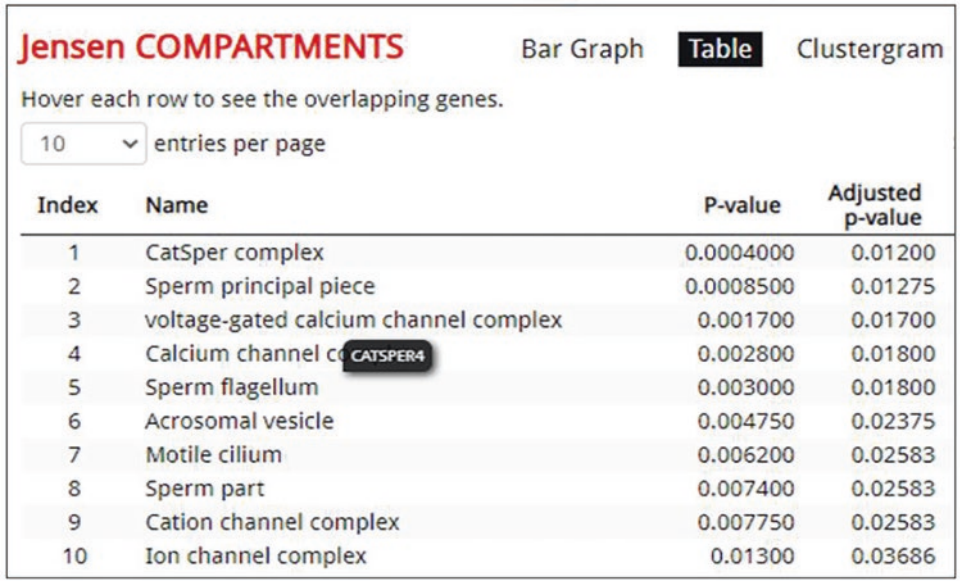
Elsevier Pathway Collection Bar Graph **Table** Clustergram

Hover each row to see the overlapping genes.

10 entries per page

Index	Name	P-value	Adjusted p-value
1	Sperm Motility Impairement in Testicular Male Infertility	e-10	1.962e-9
2	Proteins Involved in Male Infertility	3.502e-8	2.276e-7
3	Genes with Mutations Associated with Testicular Male Infertility	0.0001913	0.0008288
4	CFTR in Sperm Capacitation and Acrosome Reaction	0.007675	0.02279
5	Insulin Influence on Lipogenesis	0.008767	0.02279

Fig. 18.8 Enricher result showing CATSPER family proteins and GAPDHS involved in male infertility



Jensen COMPARTMENTS Bar Graph **Table** Clustergram

Hover each row to see the overlapping genes.

10 entries per page

Index	Name	P-value	Adjusted p-value
1	CatSper complex	0.0004000	0.01200
2	Sperm principal piece	0.0008500	0.01275
3	voltage-gated calcium channel complex	0.001700	0.01700
4	Calcium channel c	0.002800	0.01800
5	Sperm flagellum	0.003000	0.01800
6	Acrosomal vesicle	0.004750	0.02375
7	Motile cilium	0.006200	0.02583
8	Sperm part	0.007400	0.02583
9	Cation channel complex	0.007750	0.02583
10	Ion channel complex	0.01300	0.03686

Fig. 18.9 Enricher result showing CATSPER4 protein involved in sperm flagellum with *p*-value 0.003000

collected, i.e., *CatSper1*, *CatSper2*, *CatSper3*, *CatSper4*, GAPDHS, PGK2, LDHC, PGAM2, and PGM4. After this PPI network analysis was performed in STRING database, which showed the interaction between CATSPER1, CATSPER2, and CATSPER3 protein with glycolytic metabolic proteins, i.e., GAPDHS and PGK2, which clearly signifies the involvement of CATSPER protein in energy-driven pathway and downregulation of these genes will surely result in low ATP production which led to low sperm motility. To validate the PPI findings, we had gone for the pathway analysis by using the Reactome Pathway database. In that, we found the involvement of CATSPER1

protein in the lipid metabolic pathway and its interaction with the APOA2 gene with 0.78 interacting score, which clearly shows the strong association of the APOA2 gene in sperm motility. After finding the association of lipid metabolic gene in sperm motility, we again did the PPI network analysis between selected proteins (CATSPER1, CATSPER2, CATSPER3, CATSPER4, GAPDHS, PGK2, LDHC, PGAM2, and PGM4) with the APOA2 gene. In that, we find a novel protein APOB (lipid metabolic gene), showing its involvement in spermatogenesis and in sperm motility which confirms its involvement in male infertility by disrupting the metabolic pathway. Then for gene ontology, we selected Enricher and DAVID databases, in which we validated the role of the APOB gene in asthenozoospermia and in sperm motility which makes it clear that by targeting this gene in vitro, we will get a differential expression of this gene, and it can become a potential biomarker for the assessment of human sperm motility. Not only this, but we also find that except CATSPER family protein, glycolytic protein (i.e., GAPDHS) is also a contributor to male infertility, which signifies the association of metabolic genes in male infertility which need to be worked on to find stronger evidence. Lastly, CATSPER4 is the least studied protein among the other CATSPER protein, so its role is not clear yet. But in PPI results, CATSPER4 protein reported its role in sperm flagellum, and in gene ontology, the association of CATSPER4 in sperm flagellum was also found with p-value of 0.003000, which is statically strongly associated, and this tells us that this gene is needed to be studied in tail defeated spermatozoa and finding its protein structure will help us in drug development.

18.5 Conclusion

Recent findings of the mechanism(s) involved in the formation and motility of spermatozoa are still limited. Every stage of spermatogenesis involves the expression of particular gene patterns. These gene expressions are needed to study for understanding the molecular markers of germ cells at various stages of spermatogenesis. This study provided a piece of useful information and new ideas for further research on the genes involved in the formation of sperm tail and metabolic pathways involved in the regulation pattern of sperm motility and impact of ROS due to aging. This study also provided new ways and prospects for research in male contraception, diagnosis, and treatment of male infertility.

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Conflict of interest The authors declare no conflict of interest, financial, or otherwise.

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